**Biallelic loss of function variants in *SYT2* cause a treatable congenital onset presynaptic myasthenic syndrome**

Sandra Donkervoort1, Payam Mohassel1, Lucia Laugwitz2, 3, Maha S. Zaki4, Erik-Jan Kamsteeg5, Reza Maroofian6,Katherine R. Chao7, Corien C. Verschuuren-Bemelmans8, Veronka Horber3, Annemarie JM. Fock9, Riley M. McCarty1, Minal S. Jain10, Victoria Biancavilla10, Grace McMacken11, Matthew Nalls1, Nicol C. Voermans12, Hasnaa M Elbendary4, Molly Snyder13, Chunyu Cai14, Tanya Lehky15 , Valentina Stanley16, 17, Susan T. Iannaccone18, A. Reghan Foley1, Hanns Lochmüller19,20,21, Joseph Gleeson16,17, Henry Houlden6**,** Tobias B. Haack2, Rita Horvath22, Carsten G. Bönnemann1

1. Neuromuscular and Neurogenetic Disorders of Childhood Section, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD, USA

2. Institute of Medical Genetics and Applied Genomics, University of Tübingen, Tübingen, Germany

3. University Children’s Hospital, Department of Paediatric Neurology, Tübingen, Germany

4. Clinical Genetics Department, Human Genetics and Genome Research Division, National Research Centre, Cairo, Egypt

5. Department of Human Genetics, Radboud University Medical Center, Nijmegen, the Netherlands

6. Department of Neuromuscular Disorders, University College London Institute of Neurology, Queen Square, London, UK

7. Center for Mendelian Genomics, Program in Medical and Population Genetics, Broad Institute of MIT and Harvard, Boston, MA, USA

8. Department of Genetics, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

9. Department of Neurology, University of Groningen, University Medical Center Groningen, Groningen, The Netherlands

10. Rehabilitation Medicine Department, Clinical Research Center, National Institutes of Health, Bethesda, Maryland, USA.

11. Department of Neurosciences, Royal Victoria Hospital, Belfast, United Kingdom

12. Department of Neurology, Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Centre, the Netherlands

13. Department of Neurology, Children's Health, Dallas, TX, USA

14. Department of Pathology, UT Southwestern Medical Center, Dallas, TX

15. EMG Section, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, Maryland

16. Laboratory for Pediatric Brain Disease, Howard Hughes Medical Institute, University of California, San Diego, La Jolla, CA, USA

17. Rady Children's Institute for Genomic Medicine, Rady Children's Hospital, San Diego, USA

18. Department of Pediatrics, UT Southwestern Medical Center, Dallas, Texas, USA

19. Department of Neuropediatrics and Muscle Disorders, Medical Center – University of Freiburg, Faculty of Medicine, Freiburg, Germany

20. Children's Hospital of Eastern Ontario Research Institute, University of Ottawa, Ottawa, Canada

21. Division of Neurology, Department of Medicine, The Ottawa Hospital, Ottawa, Canada

22. Department of Clinical Neurosciences, University of Cambridge, Cambridge, UK

**Correspondence**
Carsten G. Bönnemann, MD
National Institute of Neurological Disorders and Stroke/NIH
Porter Neuroscience Research Center
35 Convent Drive, Bldg 35, Room 2A-116
Bethesda, MD 20892-3705
Email: carsten.bonnemann@nih.gov

**Abstract**

Synaptotagmins are integral synaptic vesicle membrane proteins that function as calcium sensors and regulate neurotransmitter release at the presynaptic nerve terminal. Synaptotagmin-2 (*SYT2),* is the major isoform expressed at the neuromuscular junction. Recently, dominant missense variants in *SYT2* have been reported as a rare cause of distal motor neuropathy and myasthenic syndrome, manifesting with stable or slowly progressive distal weakness of variable severity along with presynaptic NMJ impairment. These variants are thought to have a dominant-negative effect on synaptic vesicle exocytosis, although the precise pathomechanism remains to be elucidated. Here we report seven patients of five families, with biallelic loss of function variants in *SYT2,* clinically manifesting with a remarkably consistent phenotype of severe congenital onset hypotonia and weakness, with variable degrees of respiratory involvement, and with electrodiagnostic findings consistent with a presynaptic congenital myasthenic syndrome (CMS) in some. Treatment with an acetylcholinesterase inhibitor pursued in three patients showed clinical improvement with increased strength and function. This series further establishes *SYT2* as a CMS-disease gene and expands its clinical and genetic spectrum to include recessive loss-of-function variants, manifesting as a severe congenital onset presynaptic CMS with potential treatment implications.

**Keywords**

*SYT2*, synaptotagmins, neuromuscular junction, congenital myasthenic syndrome, presynaptic CMS

**Background**

Congenital myasthenic syndromes (CMS) are a clinical and genetically heterogeneous group of disorders characterized by impaired neuromuscular transmission (Rodriguez Cruz, Palace, & Beeson, 2018). Genetic confirmation of the CMS subtype can inform the use of available treatment options tailored to the underlying molecular mechanism; however, the considerable clinical and genetic heterogeneity of CMS contribute to delayed diagnosis. In addition, approximately 40% of patients with a CMS currently remain without a confirmed genetic diagnosis (Engel, Shen, Selcen, & Sine, 2015; Nicole et al., 2017). Variants in genes associated with presynaptic transmitter release are increasingly recognized as causes of presynaptic CMS (Engel, 2018).

Synaptotagmins, are a large family of integral membrane proteins that are essential for regulating Ca2+ mediated exocytosis (Hilbush & Morgan, 1994). Synaptotagmin-2 encoded by *SYT2* is the main isoform expressed at the presynaptic neuromuscular junction and functions as a calcium sensor for neurotransmission (Littleton, Stern, Perin, & Bellen, 1994; Mackler, Drummond, Loewen, Robinson, & Reist, 2002; Pang et al., 2006). SYT2 is a synaptic vesicle protein that contains two calcium binding domains, C2A (AA 141-242) and C2B (AA 272-375), and regulates neurotransmitter release through mediating fast synaptic vesicle exocytosis (Littleton et al., 1994; Mackler et al., 2002; Pang et al., 2006). At this time, three dominantly actingmissense variants impacting the *SYT2* C2B domain have been reported as a rare cause of distal motor neuropathy and myasthenic syndrome. Patients clinically manifest with a relatively stable or slowly progressive distal weakness of variable severity with physiologic evidence of presynaptic NMJ impairment, responsive to 3,4 diaminopyridine treatment in some patients (Herrmann et al., 2014; Montes-Chinea et al., 2018; Whittaker et al., 2015). These *SYT2* variants are thought to impair Ca2+ binding and disrupt synaptic transmission in a dominant-negative manner, however, their detailed mode of action remains to be fully explored. Recently, one patient with severe presynaptic CMS and denervation atrophy was reported to have billalic variants in *SYT2* (Maselli, van der Linden, & Ferns, 2020) .

In this study we report seven patients of five independent families with biallelic loss of function (lof) variants in *SYT2*,clinically manifesting with a remarkably consistent phenotype of severe congenital onset hypotonia and profound weakness, with minimal attainment of motor milestones and with variable degrees of respiratory involvement. Treatment withan acetylcholinesterase inhibitor in three patients showed clinical improvement. Taken together, our data introduce recessive loss of function variants in *SYT2* as an important mutational mechanism, establish the role of SYT2 in neuromuscular disease, and expand its clinical spectrum to a severe congenital onset phenotype that is potentially treatable.

**Methods**

*Patient Recruitment and Sample Collection*

Patients presented with a history of congenital onset muscle weakness and underwent detailed clinical examination. DNA, tissues including muscle, and medical records were obtained based on standard procedures. For research studies, written informed consent and age appropriate assent was obtained. This study was approved by the NIH, National Institute of Neurological Disorders and Stroke (NINDS), Institutional Review Board (Protocol 12‐N‐0095), the Yorkshire & The Humber - Leeds Bradford Research Ethics Committee (13/YH/0310). P3 was identified through GeneMatcher (Sobreira, Schiettecatte, Valle, & Hamosh, 2015).

*Genetic Analysis*

Trio whole exome sequencing was performed in all patients. Variants were confirmed by Sanger sequencing. For P1 exon-level oligo array comparative genomic hybridization (CGH) was performed for *SYT2.* Detailed methods are provided in Supplementary material 1.

*Motor Assessments*

P1’s motor capacity and strength were evaluated at baseline (visit 1) and at 13 months after treatment initiation (visit 2). Motor capacity was measured using the Motor Function Measure-32 (MFM32), comprised of three domains (D1: standing and transfers; D2: axial and proximal motor function; and D3: distal motor function) with results expressed as percentages of total and domain scores. A higher score indicates better performance. Motor strength was quantitatively measured using a handheld dynamometer (MicroFET®2 [hogganhealth.net]) for knee, hip, and elbow strength with distal strength measured using grip and pinch dynamometers (MyoGrip and MyoPinch (Ateliers Laumonier, France)(Hogrel, 2015). Handheld dynamometry results were expressed as the percentage of the highest value compared to age/weight/sex-based norms (Beenakker, van der Hoeven, Fock, & Maurits, 2001), and distal strength results were expressed as the percentage of the highest value compared to age-based norms (Annoussamy et al., 2019). A single pediatric physical therapist completed all motor capacity and strength assessments at both visits using standard positions and instructions.

**Results**

*Clinical Characteristics*

Patient 1 (P1) is a 15-year-old Caucasian female born to consanguineous parents. Pregnancy was notable for reduced fetal movements. At birth she was noted to have hypotonia, abnormal wrist and ankle positioning due to joint laxity, a high-arched palate, and a weak cry. She was diagnosed with failure to thrive requiring nasogastric (NG) tube feeding which was subsequently converted to gastrostomy tube. Motor milestones were delayed. She was able to sit independently at the age of two years but has not attained the ability to roll, stand or walk. She has a history of structural cardiac disease with hypertrophic obstructive cardiomyopathy and mitral valve stenosis requiring cardiac reconstructive surgery in the first year of life. Additionally, she has a history of eye deviation requiring corrective surgery. Progressive scoliosis was noted at an early age for which she underwent corrective surgery at age 11 years (Figure 1). Examination at age 14 years revealed significant axial weakness [Medical Research Council (MRC) grade 2/5] and proximal weakness (MRC grade 2-3/5) more than distal weakness (MRC grade 4-/5). Sensation appeared normal. Reflexes were absent throughout the bilateral upper and lower extremities; however, post-exercise facilitation of biceps reflex was noted bilaterally. Bilateral upper and lower facial weakness was noted. Extraocular movements were notable for slow saccades, and bilateral adduction and abduction restriction and supraduction limitations that were still present post corrective surgery. Cognition was normal. Forced Vital Capacity (FVC) was 40% predicted. Brain magnetic resonance imaging (MRI) at age 14 years revealed mild cerebral atrophy, including thinning of the corpus callosum (Figure 1f). Muscle ultrasound at age 14 years showed a mixed granular and streaky pattern of increased echogenicity with rare fasciculations (Figure 1g, h).

~~A~~ Treatment ~~trial~~ of pyridostigmine was initiated in P1 at age 14 years. Her family reported an improvement in her function over the three months following the start of pyridostigmine. Subsequently, she has continued to improve in some functional domains but at a slower pace. Between visit 1 and visit 2, 12 months after initiation of pyridostigmine, the patient improved in the relative strength of all muscle groups between 1.2% and 10.8%, except for the right elbow extensors which decreased by 1.7%. In particular, the patient improved in the relative strength of both grip and pinch between 1.8% and 7.8%. The total MFM score between visit one and visit two increased by seven points, from 26 to 33, out of a total of 96 possible points, with an increase of five points considered reaching minimal clinically important difference (MCID) (Vuillerot et al., 2012). D1 remained stable, while scores on D2 and D3 increased from visit 1 to visit 2. 3,4-Diaminopyridine (3,4-DAP) has been successfully used in some patients with CMS. However, given P1’s cardiac disease and baseline long QT interval, this medication was not initiated.

We subsequently identified six additional patients, three males and three females of four independent families. The clinical presentation is summarized in Table 1, with ages ranging from 25 months to 15 years. All of the parents reported no clinical symptoms, and family history was significant for consanguinity in all four families (Figure 3a). All patients presented with a remarkably consistent phenotype of congenital onset severe hypotonia significant generalized weakness noted at birth. Patients had attainment of only minimal motor milestones, with maximal motor milestones ranging from minimal head control in two patients, to independent sitting in four patients. At the time of the last examination, all patients were found to have significant generalized weakness. Slow and limited extraocular movements were seen in five patients. Pulmonary function ranged from normal to requiring full-time ventilation (P5). Similar to P1, the brain MRI performed in P6 also revealed a thin corpus callosum. Additionally, P6 had evidence of mild vermian hypoplasia. The brain MRI in P2 and P3 was normal. None of the patients had a history of seizures, cataracts, hearing loss or cognitive involvement. Unlike P1, cardiac involvement was not observed in P2-7. A trial of pyridostigmine was initiated in P4 without evidence of a clinical response. Both siblings in Family 2 (P6 and P7) initiated neostigmine (an acetylcholinesterase inhibitor) and subsequently reported an increase in overall stamina and motor activity, and both patients were less susceptible to chest infections. On strength examination, P6 and P7 were noted to have increased head control and increased movement against gravity since initiation of neostigmine.

*Electrodiagnostic Findings*

A subset of patients had electrodiagnostic studies performed. These studies showed normal sensory responses. Motor nerve conduction studies universally revealed significantly reduced compound muscle action potential (CMAP) amplitudes and normal nerve conduction velocities. EMG showed reduced recruitment; however, motor unit action potential (MUAP) morphology varied with some patients having short duration, low amplitude MUAPs while others having large amplitude and long duration units. Abnormal spontaneous activity, in the form of positive sharp waves and fibrillation potentials were present in some but not all patients. In those that had repetitive nerve stimulation (RNS) studies, low frequency (3-5 Hz) stimulation resulted in significant decremental responses pointing to myasthenic syndrome. Post-exercise facilitation could not be reliably observed at the very low CMAP amplitudes.

*Histological Characteristics*

Muscle biopsies were performed in two patients (P1 and P2). Common findings include variation in fiber size (Figure 2a) with occasional angular fibers. Gömöri trichome staining in P1 demonstrated occasional fibers with fuscinophilic sarcoplasmic and perinuclear inclusions (Figure 2b) which were NADH positive (Figure 2c) suggestive of tubular aggregates. Non-specific esterase staining in P1 highlighted the tubular aggregates and appeared to show segmented NMJs. A rare angular, atrophic fiber is also noted (Figure 2d).

*Genetic Findings*

Trio whole exome sequencing for P1 was unrevealing. Subsequent analysis of the data for copy number variants in P1 identified an apparent homozygous loss of coverage of a portion of *SYT2* in P1 compared to controls, with each parent showing apparent heterozygosity for this deletion (Figure 3b). This was confirmed through targeted array CGH analysis with exon-level resolution revealed a homozygous deletion including at least exons 3-9 of the *SYT2* gene in P1 (arr[GRCh37] 1q32.1(202565663\_202574633)x0

Using whole exome sequencing we identified three homozygous bi-allelic loss of function variants in *SYT2* (ENST00000367267in six patients of four families:

P2:c.[927C>A];[927C>A];p.[(Tyr309\*)];[(Tyr309\*)], P3, P6 and P7: c.[725dup]; [725dup]; p.[(Val243Glyfs\*13)]; [(Val243Glyfs\*13)], P4 and P5: c.[805G>T]; [805G>T]; p.[(Glu269\*)];[(Glu269\*)]. The SYT2 loss of function variants are scattered throughout the gene and do not seem to cluster in a specific domain (Figure 3c). Variants are classified as pathogenic (Strong: PS3, PS4, Moderate: PM2, PM3, Supporting: PP3, PP4) based on the 2015 American College of Medical Genetics and Genomics-Association for Molecular Pathology (ACMG-AMP) guideline for variant interpretation (Richards et al., 2015).

**Discussion**

To date, three independent dominantly acting heterozygous variants in *SYT2* have been reported in five families as a rare cause of slowly progressive distal motor neuropathy and myasthenic syndrome (Herrmann et al., 2014; Montes-Chinea et al., 2018; Whittaker et al., 2015). Here we report a series of seven patients with recessive loss of function variants in *SYT2,* clinically manifesting with a severe presynaptic CMS. Overall, we establish the role of *SYT2* in neuromuscular disease and expand its mutational mechanisms to include biallelic loss of function variants and its clinical spectrum to include a severe congenital disorder with predominant NMJ dysfunction.

Clinically, SYT2*-*deficient patients presented at birth with severe hypotonia and profound weakness significantly interfering with the attainment of motor milestones. The additional findings of fasciculations (P1) and apparent neurogenic MUAPs on EMG may phenotypically resemble lower motor neuropathy/neuronopathy (e.g. spinal muscular atrophy (SMA) or rarer causes of congenital onset SMA with respiratory failure such as those caused by variants in *IGHMBP2* and *LAS1L* (Butterfield et al., 2014; Grohmann et al., 2001))*.* The lack of fiber type grouping, a histologic hallmark of chronic neurogenic disease, as well as the overall mild changes on muscle biopsy compared to the profound level of weakness would not be consistent with a significant neuronopathy as the driver of the weakness, but would be more compatible with a dysfunctional NMJ. Additional histological clues are the tubular aggregates that were seen in in P1’s muscle biopsy, which have been reported in other forms of CMS. Namely CMS due to recessive mutations in *GFPT1* or *DPAGT1* which encode enzymes in protein glycosylation pathways as well as tubular aggregate myopathies caused by impaired intracellular Ca2+ handling (Bohm & Laporte, 2018; Chevessier et al., 2005). Post-exercise facilitation on RNS in P4 is consistent with this plausible pathophysiologic mechanism. Thus, we postulate that the profound muscle weakness seen in our SYT2-deficient patients is largely driven by significantly impaired presynaptic neuromuscular junction transmission, consistent with SYT2’s role, with an additional element of secondary axonal degeneration. Dedicated morphological analysis of the NMJ, the gold standard for evaluation of such disorders, was not available, however, and limited our ability to definitively characterize *SYT2-*related disease.

Brain MRI imaging was performed in four patients (P1, P2, P3 and P6). P1 and P6 had evidence of mild cerebral atrophy, including thinning of the corpus, and P6 was also found to have a thin corpus callosum; the brain MRI in P2 and P3 was normal. Unfortunately, imaging was not available in the remaining patients. It remains unclear whether the brain findings are related to the SYT2-deficiency at this time, which however remains a possibility as presynaptic CMS on the whole are more likely to also include central nervous system manifestations. Thus, as additional patients are being recognized, this may very well be a more consistent finding of the SYT2-deficiency clinical spectrum. At this time, P1 is the only patient with cardiac involvement, it remains unclear whether this is related to the loss of SYT2, or whether there may be a second genetic etiology at play. It is also notable that arthrogryposis, congenital brain malformations and epilepsy were not reported, which clinically distinguishes SYT2-deficiency from the various congenital motor neuronopathies including pontocerebellar hypoplasias, and SMA with progressive myoclonic epilepsy (SMA-PME). Muscle fatiguability, a hallmark of CMS, was difficult to assess in our SYT2*-*deficient cohort due to the severe weakness observed in all patients. Involvement of the ocular and facial muscles was seen in our patients. The profound congenital presentation is not unusual for CMS. More recently, patients with variants in presynaptic genes impacting neurotransmitter release were found to have more significant multisystemic involvement, including central nervous system. These proteins are often involved in SNARE-mediated vesicle fusion at the presynaptic nerve terminal with pathogenic variants resulting in a wide clinical spectrum ranging from a severe presynaptic CMS similar to SYT2-deficient patients, as seen in patients with pathogenic variants in *VAMP1*, to those with multisystemic involvement including **cortical hyperexcitability, ataxia, and intellectual disability as seen in patients with** pathogenic variants **in *SNAP25B*.** (Rodriguez Cruz et al., 2018) (Salpietro et al., 2017) (Shen, Selcen, Brengman, & Engel, 2014).

Treatment options are available for selected CMS, depending on the individual molecular mechanism of NMJ dysfunction (Engel et al., 2015). Thus, an understanding of the underlying disease mechanism is imperative for a rational therapeutic choice to be made and to avoid inadvertent worsening of the condition. Given SYT2’s role at the presynaptic NMJ, a trial of pyridostigmine was initiated in P1 following her genetic confirmatory diagnosis at age 14 years. P1 has a history of cardiac disease and baseline long QT interval; therefore, a trial of 3,4-DAP was decided against, given the potential of cardiac side effects. The patient and her parents reported an increase in muscle strength and stamina, with an improvement in daily activities. At her follow up 12 months after starting pyridostigmine, motor improvements were observed with repeat manual muscle testing and quantified through various physical therapy measures, with an increase in total MFM score reaching the MCID (Vuillerot et al., 2012). The initiation of therapeutic intervention in P1 was delayed given a lack of a confirmed genetic diagnosis, and earlier treatment may have resulted in a larger clinical response. A therapeutic reponse to an acetylchoinesterase was not a universal finding in SYT2-deficient patients, and in particular, P4 did not note any clinical improvement with a pyridostigmine trial.

SYT2 is part of the large synaptotagmin family, which is made up of synaptic vesicle membrane proteins that function as calcium sensors and regulate neurotransmitter release at the presynaptic nerve terminal. SYT2 shares the highest sequence homology with SYT1, and they are often co-expressed with possible functional redundancy (Marqueze et al., 1995; Ullrich et al., 1994). In fact, overexpression of Syt2 in cells from Syt1 null mice was able to partially rescue the impaired exocytosis (Nagy et al., 2006). Syt2 null mice were found to have moderate increase of Syt1 in the spinal cord, which was not seen in the brain (Pang et al., 2006). Furthermore, developmental expression studies suggest that SYT1 is essential in pre- and early postnatal neuromuscular transmission, with an observed delayed expression and subsequent isoform switch to SYT2 (Berton, Iborra, Boudier, Seagar, & Marqueze, 1997; Kochubey, Babai, & Schneggenburger, 2016). Interestingly, *de novo* dominant variants in *SYT1* were recently reported to cause a rare form of neurodevelopmental disorders (Baker et al., 2018). Unfortunately, access to patient tissue for further validation work is challenging as SYT2 is not expressed in human fibroblasts. Thus, we were unable to confirm a complete absence of SYT2 in our patients and explore a potential compensatory upregulation of SYT1.

In our patients we see maximal manifestation of the phenotype at birth, without overt subsequent disease progression, indicating a possible phase of prenatal progression and a developmental role for SYT2 in humans. In this way, SYT2 deficiency in our patients may resemble the phenotype previously reported in Syt2-deficient mice, which are normal at birth but subsequently developed a rapid progressive motor dysfunction due to impaired synaptic transmission, with complete paralysis resulting in lethality at approximately three weeks of age (Pang et al., 2006). In addition, Drosophila synaptotagmin null mutants show early lethality. They have been reported to survive to adulthood, however, when placed on food, hence requiring limited movement, and they subsequently display motor defects with impaired synaptic transmission (Loewen, Mackler, & Reist, 2001).

Coincidentally, the SYT2-deficient mouse model was generated by replacing exon 2 through 7 with a knock-in LacZ sequence, creating a null allele (Pang et al., 2006), which has a remarkable resemblance to P1 genetically (homozygous exon 3-9 deletion) and phenotypically. In this context it is notable that in humans *SYT2* also does not seem very tolerant of loss of function variants. Only five null alleles are reported in gnomAD, all in heterozygous state only (probability of being loss-of-function intolerant (pLI) score of 0.98 and observed / expected*(*oe*)* score of 0.06). However, haploinsufficiency of *SYT2* does not appear to cause disease, as the heterozygous carrier parents in this cohort did not report any symptoms. While the exact pathogenic mechanism of the previously reported dominant *SYT2* missense variants remains largely unknown, they all impact the SYT2 calcium binding domains and are thought to impair Ca2+ binding in a dominant-negative manner, resulting in a loss of synaptic transmission beyond the haploinsufficiency state.

While the majority of *SYT2* loss of function variants reported here can be accurately identified through standard next generation-based sequencing platforms, large deletions are typically missed. The partial deletion of *SYT2* (exon 3-9) identified in P1 was initially missed on whole exome sequencing but was subsequently identified through research-based reanalysis of the WES data for copy number variants. Therefore, clinical recognition of this newly described SYT2-deficient phenotype is essential in facilitating appropriate diagnostic testing. Our series further establishes *SYT2* as a CMS disease gene and expands its genetic spectrum to include a recessively acting loss of function mutations and expands its clinical spectrum to include severe congenital onset presynaptic CMS. Although not universal, some patients may benefit from CMS therapies directed toward improving the function of the presynaptic neuromuscular junction.

**Acknowledgments**

We thank the patients and their families for participating in our research study and Christopher Mendoza, Christine Jones, and Gilberto (“Mike”) Averion for their help in clinic. We also thank the Genome Aggregation Database (gnomAD) and the groups that provided exome and genome variant data to this resource. A full list of contributing groups can be found at <https://gnomad.broadinstitute.org/about>.

**Conflict of Interest**

The authors have no conflicts of interests to report.

**Author contribution**

Sandra Donkervoort, Payam Mohassel, Lucia Laugwitz, Rita Horvath, Tobias B. Haack, Carsten G. Bönnemann contributed to the concept, design, data collection, and manuscript writing. Maha S. Zaki, Corien C. Verschuuren-Bemelmans, Veronka Horber, Annemarie JM. Fock, Minal S. Jain, Victoria Biancavilla, Grace McMacken, Matthew Nalls, Nicol C. Voermans, Hasnaa M Elbendary, Molly Snyder, Chunyu Cai, Tanya Lehky, Susan T. Iannaccone, A. Reghan Foley contributed patient identification and characterization. Katherine R. Chao, Lucia Laugwitz, Erik-Jan Kamsteeg, Reza Maroofian, Riley M. McCarty, Valentina Stanley, Joseph Gleeson, Hanns Lochmüller, Henry Houlden**,** Tobias B. Haack contributed molecular data collection and interpretation.  All authors reviewed the manuscript.

Posey

assisted with molecular data collection and interpretation

**Data Sharing**

The data that support the findings will be available in dbGAP and ClinVAR.

**Funding**

The work in C.G. Bönnemann’s laboratory is supported by intramural funds from the NIH National Institute of Neurological Disorders and Stroke. RH is a Wellcome Trust Investigator (109915/Z/15/Z), who receives support from the Medical Research Council (UK) (MR/N025431/1), the European Research Council (309548), the Wellcome Trust Pathfinder Scheme (201064/Z/16/Z) and the Newton Fund (UK/Turkey, MR/N027302/1). HL is the recipient of a Canadian Institutes of Health Research Foundation Grant (CIHR FDN-167281). Sequencing and analysis were provided by the Broad Institute of MIT and Harvard Center for Mendelian Genomics (Broad CMG) and was funded by the National Human Genome Research Institute, the National Eye Institute, and the National Heart, Lung and Blood Institute grant UM1 HG008900 and in part by National Human Genome Research Institute grant R01 HG009141.

**Figure 1:** Clinical presentation (a) P1 with evidence of overall reduced muscle bulk, facial weakness, (b) scoliosis, contractures of the elbows and fingers with neuropathic looking hands (c) and (d) feet. (e) Patient P6 with evidence of reduced muscle bulk and severe facial weakness. (f) Brain MRI of P1 at age 14 years revealed thinning of the corpus callosum (exact midline section not available due to artifact related to to dental braces). (g and h) Muscle ultrasound in P1 of the right deltoid (g) which is atrophic and with a moderate to severely increased echogenicity and the right rectus femoris (h) a mild to moderately increased echogenicity. In both muscles (g and h) the echogenicity had a mixed pattern (granular and streaky in qualitative appearance)

**Figures 2:** Quadriceps muscle biopsy of P1 age 4 years. (a) Variation in fiber size on hematoxylin and eosin (H&E) stain. (b) Inclusions suggestive of tubular aggregates on Gömöri trichome (60x). (c) Nicotinamide adenine dinucleotide reductase NADH (60x) positive staining seen in the tubular aggregate-like inclusions. (d) Esterase highlighting the neuromuscular junction (black arrow), tubular aggregates (blue arrow), and an angulated atrophic fiber (white arrow).

**Figures 3:** (a) Pedigrees of the patients with loss of function variants in the *SYT2* gene. (b) Copy number variant analysis of the whole exome sequencing data identified an apparent homozygous loss of coverage of a portion of *SYT2* in P1 (blue) compared to control (grey), with each parent showing apparent heterozygosity for this deletion (purple). (c) New and reported human *SYT2* variants. The numbered, blue rectangles represent exons (RefSeq isoform NM\_177402.5), while the orange ovals represent C2 calcium-binding protein domains. The slimmer blue rectangles represent untranslated region. The variants on the top half are reported in this series, while the variants on the bottom half have previously been reported. Variants in black represent dominant variants, while the ones in red represent recessive variants. The dotted line found on the top half of the figure represents a large deletion (exons 3 – 9). (d) Normed quantitative strength measurements assessed using handheld dynamometry for P1 at visit 1 and visit 2 (left) and Radar graph representingMFM domain and total scores for P1 at visit 1 and visit 2, with the outer bounds of the chart representing 100% of total possible points in each domain (right). D1: standing and transfers; D2: axial and proximal motor function; and D3: distal motor function.

**Table 1:** Clinical details of the *SYT2* patients.

**References**

Annoussamy, M., Lilien, C., Gidaro, T., Gargaun, E., Che, V., Schara, U., . . . Servais, L. (2019). X-linked myotubular myopathy: A prospective international natural history study. *Neurology, 92*(16), e1852-e1867. doi:10.1212/WNL.0000000000007319

Baker, K., Gordon, S. L., Melland, H., Bumbak, F., Scott, D. J., Jiang, T. J., . . . Raymond, F. L. (2018). SYT1-associated neurodevelopmental disorder: a case series. *Brain, 141*(9), 2576-2591. doi:10.1093/brain/awy209

Beenakker, E. A., van der Hoeven, J. H., Fock, J. M., & Maurits, N. M. (2001). Reference values of maximum isometric muscle force obtained in 270 children aged 4-16 years by hand-held dynamometry. *Neuromuscul Disord, 11*(5), 441-446. doi:10.1016/s0960-8966(01)00193-6

Berton, F., Iborra, C., Boudier, J. A., Seagar, M. J., & Marqueze, B. (1997). Developmental regulation of synaptotagmin I, II, III, and IV mRNAs in the rat CNS. *J Neurosci, 17*(4), 1206-1216. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/9006966>

Butterfield, R. J., Stevenson, T. J., Xing, L., Newcomb, T. M., Nelson, B., Zeng, W., . . . Swoboda, K. J. (2014). Congenital lethal motor neuron disease with a novel defect in ribosome biogenesis. *Neurology, 82*(15), 1322-1330. doi:10.1212/WNL.0000000000000305

Engel, A. G. (2018). Congenital Myasthenic Syndromes in 2018. *Curr Neurol Neurosci Rep, 18*(8), 46. doi:10.1007/s11910-018-0852-4

Engel, A. G., Shen, X. M., Selcen, D., & Sine, S. M. (2015). Congenital myasthenic syndromes: pathogenesis, diagnosis, and treatment. *Lancet Neurol, 14*(5), 461. doi:10.1016/S1474-4422(15)00010-1

Grohmann, K., Schuelke, M., Diers, A., Hoffmann, K., Lucke, B., Adams, C., . . . Hubner, C. (2001). Mutations in the gene encoding immunoglobulin mu-binding protein 2 cause spinal muscular atrophy with respiratory distress type 1. *Nat Genet, 29*(1), 75-77. doi:10.1038/ng703

Herrmann, D. N., Horvath, R., Sowden, J. E., Gonzalez, M., Sanchez-Mejias, A., Guan, Z., . . . Zuchner, S. (2014). Synaptotagmin 2 mutations cause an autosomal-dominant form of lambert-eaton myasthenic syndrome and nonprogressive motor neuropathy. *Am J Hum Genet, 95*(3), 332-339. doi:10.1016/j.ajhg.2014.08.007

Hilbush, B. S., & Morgan, J. I. (1994). A third synaptotagmin gene, Syt3, in the mouse. *Proc Natl Acad Sci U S A, 91*(17), 8195-8199. doi:10.1073/pnas.91.17.8195

Hogrel, J. Y. (2015). Grip strength measured by high precision dynamometry in healthy subjects from 5 to 80 years. *BMC Musculoskelet Disord, 16*, 139. doi:10.1186/s12891-015-0612-4

Kochubey, O., Babai, N., & Schneggenburger, R. (2016). A Synaptotagmin Isoform Switch during the Development of an Identified CNS Synapse. *Neuron, 91*(5), 1183. doi:10.1016/j.neuron.2016.08.024

Littleton, J. T., Stern, M., Perin, M., & Bellen, H. J. (1994). Calcium dependence of neurotransmitter release and rate of spontaneous vesicle fusions are altered in Drosophila synaptotagmin mutants. *Proc Natl Acad Sci U S A, 91*(23), 10888-10892. doi:10.1073/pnas.91.23.10888

Loewen, C. A., Mackler, J. M., & Reist, N. E. (2001). Drosophila synaptotagmin I null mutants survive to early adulthood. *Genesis, 31*(1), 30-36. doi:10.1002/gene.10002

Mackler, J. M., Drummond, J. A., Loewen, C. A., Robinson, I. M., & Reist, N. E. (2002). The C(2)B Ca(2+)-binding motif of synaptotagmin is required for synaptic transmission in vivo. *Nature, 418*(6895), 340-344. doi:10.1038/nature00846

Marqueze, B., Boudier, J. A., Mizuta, M., Inagaki, N., Seino, S., & Seagar, M. (1995). Cellular localization of synaptotagmin I, II, and III mRNAs in the central nervous system and pituitary and adrenal glands of the rat. *J Neurosci, 15*(7 Pt 1), 4906-4917. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/7623121>

Maselli, R. A., van der Linden, H., Jr., & Ferns, M. (2020). Recessive congenital myasthenic syndrome caused by a homozygous mutation in SYT2 altering a highly conserved C-terminal amino acid sequence. *Am J Med Genet A*. doi:10.1002/ajmg.a.61579

Montes-Chinea, N. I., Guan, Z., Coutts, M., Vidal, C., Courel, S., Rebelo, A. P., . . . Saporta, M. A. (2018). Identification of a new SYT2 variant validates an unusual distal motor neuropathy phenotype. *Neurol Genet, 4*(6), e282. doi:10.1212/NXG.0000000000000282

Nagy, G., Kim, J. H., Pang, Z. P., Matti, U., Rettig, J., Sudhof, T. C., & Sorensen, J. B. (2006). Different effects on fast exocytosis induced by synaptotagmin 1 and 2 isoforms and abundance but not by phosphorylation. *J Neurosci, 26*(2), 632-643. doi:10.1523/JNEUROSCI.2589-05.2006

Nicole, S., Azuma, Y., Bauche, S., Eymard, B., Lochmuller, H., & Slater, C. (2017). Congenital Myasthenic Syndromes or Inherited Disorders of Neuromuscular Transmission: Recent Discoveries and Open Questions. *J Neuromuscul Dis, 4*(4), 269-284. doi:10.3233/JND-170257

Pang, Z. P., Melicoff, E., Padgett, D., Liu, Y., Teich, A. F., Dickey, B. F., . . . Sudhof, T. C. (2006). Synaptotagmin-2 is essential for survival and contributes to Ca2+ triggering of neurotransmitter release in central and neuromuscular synapses. *J Neurosci, 26*(52), 13493-13504. doi:10.1523/JNEUROSCI.3519-06.2006

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., . . . Committee, A. L. Q. A. (2015). Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. *Genet Med, 17*(5), 405-424. doi:10.1038/gim.2015.30

Rodriguez Cruz, P. M., Palace, J., & Beeson, D. (2018). The Neuromuscular Junction and Wide Heterogeneity of Congenital Myasthenic Syndromes. *Int J Mol Sci, 19*(6). doi:10.3390/ijms19061677

Salpietro, V., Lin, W., Delle Vedove, A., Storbeck, M., Liu, Y., Efthymiou, S., . . . Houlden, H. (2017). Homozygous mutations in VAMP1 cause a presynaptic congenital myasthenic syndrome. *Ann Neurol, 81*(4), 597-603. doi:10.1002/ana.24905

Shen, X. M., Selcen, D., Brengman, J., & Engel, A. G. (2014). Mutant SNAP25B causes myasthenia, cortical hyperexcitability, ataxia, and intellectual disability. *Neurology, 83*(24), 2247-2255. doi:10.1212/WNL.0000000000001079

Sobreira, N., Schiettecatte, F., Valle, D., & Hamosh, A. (2015). GeneMatcher: a matching tool for connecting investigators with an interest in the same gene. *Hum Mutat, 36*(10), 928-930. doi:10.1002/humu.22844

Ullrich, B., Li, C., Zhang, J. Z., McMahon, H., Anderson, R. G., Geppert, M., & Sudhof, T. C. (1994). Functional properties of multiple synaptotagmins in brain. *Neuron, 13*(6), 1281-1291. Retrieved from <https://www.ncbi.nlm.nih.gov/pubmed/7993622>

Vuillerot, C., Payan, C., Girardot, F., Fermanian, J., Iwaz, J., Berard, C., . . . Group, M. F. M. S. (2012). Responsiveness of the motor function measure in neuromuscular diseases. *Arch Phys Med Rehabil, 93*(12), 2251-2256 e2251. doi:10.1016/j.apmr.2012.05.025

Whittaker, R. G., Herrmann, D. N., Bansagi, B., Hasan, B. A., Lofra, R. M., Logigian, E. L., . . . Lochmuller, H. (2015). Electrophysiologic features of SYT2 mutations causing a treatable neuromuscular syndrome. *Neurology, 85*(22), 1964-1971. doi:10.1212/WNL.0000000000002185